THE MIGRATION OF THE GERM CELLS IN AMIURUS NEBULOSUS.

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A study of the germ cells in *Amiurus nebulosus* was undertaken with the hope of determining their origin, but because of the difficulties in distinguishing them in the younger stages of the embryo and the limited amount of time in which this work was done, it was necessary to limit the subject to a study of their number and migration after they are clearly distinguishable.

The material was fixed in either Zenker's fluid or bichromate acetic. The two fluids appear to give equally good results. All sections were cut 10 microns thick and stained with Heidenhain's iron-alum hematoxylin followed by Congo red or with Mayer's hæmalum followed by Orange G.

The origin and migration of the sex cells and the formation of the germ gland in teleosts have been studied in a number of species by several investigators. Some have concluded that the germ cells are all or in part transformed epithelial cells; others believe they are never a part of the body tissues but are derived from undifferentiated cells in the very early stages of development and later migrate into the sex gland where by division, they give rise to oogonia and spermatogonia.

Nussbaum ('80) in working on the trout, found no evidence of any epithelial cells transformed into germ cells. At a certain stage in development, the germ cells divide and form little groups or nests of cells. He thought these could not be transformed epithelial cells because if the cells in the groups are of epithelial origin, then why should they be in groups, and also, if epithelial cells are continually being transformed into germ cells, then why, when these smaller cells appear in groups, do the larger cells which were in stages immediately preceding so suddenly and completely disappear? Also he found if there was a large number of cells in a nest the individual cells were smaller than those in nests where there were but a few cells. He thought the size of

the individual cells in a nest in proportion to the size of the nest, good proof that no epithelial cells are transformed into germ cells. The nests of cells are at first some distance apart and become nearer together as the cells increase in number by division —not as new cells are formed from epithelial cells.

MacLeod ('81) in *Hippocampus* and *Belone acus* observed that the sex cells appear in the somatopleure and splanchopleure rather late in development and thought them to be differentiated peritoneal cells. He thought the genital fold originates in a group of germ cells on the surface of the epithelium.

Hoffmann ('86) studied salmon embryos. According to him the genital glands have their origin in the primary germ cells which are first found in the median and dorsal part of the mesentery and laterally to the body wall. He could see nothing more in these germ cells than transformed and highly differentiated peritoneal cells which in the youngest stages of development increase in number by mitosis. Later these cells come to lie laterally from the mesentery and extend into the body cavity as a small fold. The cells increase in number by division and transformation of more peritoneal cells. Nests of germ cells are thus formed. Later each cell becomes surrounded by follicle cells. He could not say whether these follicle cells are connective tissue cells or of epithelial origin, *i. e.*, from a germinal epithelium. However, he inclined to the latter because the follicle cells appear before a connective tissue stroma is differentiated.

Böhi ('04) worked on the trout and the salmon. In the trout, twenty-five days after fertilization and six days later in the salmon, he found large cells among the larger epithelial cells in both the somatopleure and splanchopleure. The cells have very large nuclei but no nucleoles. The cytoplasm stains lightly and is without definite structure. The nuclear content is granular and hence takes little of the stain. The cells are more oval or ovoid in younger embryos and rounder in older ones. There is a diminution in volume before division begins. There is a definite relation between the size of the nucleus and the size of the cell—the large cells always have the larger nuclei. In two trout embryos of twenty-five days he found in the lateral plate four and six germ cells respectively. In salmon embryos of

thirty-one days he found germ cells in the same position. After the cœlome is formed he found the cells in both somatopleure and splanchnopleure—in one embryo 13 in the somatopleure, 4 in the splanchnopleure and 3 between these layers.

In salmon embryos the number of germ cells remains constant up to 185 days after fertilization. But this number varies from 20 to 54. In an embryo of 154 days he found 46 germ cells, in one of 185 days, 234 cells, in one of 199 days, 373 cells. The most posterior germ cell is six or seven somites anterior to the anus. As the embryo develops the nuclei enlarge to some extent so that the ratio of the diameter of the nucleus to that of the cell becomes somewhat greater. The nucleus has a sharper contour, loses its granular appearance and shows small chromatin bodies. From 46 to 50 days after fertilization the first small nucleoles appear, later they are larger, stain more deeply and increase in number.

The genital ridge arises as a discontinuous thickening of the cœlomic epithelium in the region of a germ cell. The thickenings unite and others are formed anteriorly and posteriorly. The uniting of these thickenings takes place at about 60 days. By 82 days the ridge is continuous in the gonal part of the embryo and is represented in the progonal and epigonal region by separate thickenings. The end of the genital ridge is posterior to the anus in one salmon embryo. The genital fold rises discontinuously. The cœlome cells of the genital ridge pull apart and leave a space in which there is a fluid. This fold extends progonally and epigonally and ends in the genital ridge.

In agreement with Nussbaum and Jungersen, Böhi found the genital fold to be composed of epithelial cells and not as MacLeod had observed it—a group of germ cells on the surface of the epithelium. Böhi believed the cœlome cells to give rise to (*a*) indifferent cells, (*b*) follicle cells, (*c*) germ cells. The cells on the sides of the genital fold gradually become transformed into germ cells. This changing of epithelial cells into germ cells lasts for only a short time and in embryos of 277 days no more transition cells can be found. The epithelial cells next to the germ cells are converted into follicle cells by pressure of the germ cells against them. According to Böhi some of the germ cells are

differentiated early in development while others arise from epithelial cells after the genital fold is formed.

Eigenmann ('92) in studying *Micrometrus aggregatus* found that the sex cells first become conspicuous in the mesoblast at a stage before any protovertebræ are formed. They can be seen in earlier stages but do not stand out so prominently from the other cells. Judging from their size they are probably segmentation cells of the fifth generation. The sex cells can first be distinguished about the time the blastopore closes. The earliest ones are before the mesoderm is split off from the entoderm. They differ from the surrounding cells in having well-defined rounded outlines and in the distribution of the chromatin in the nucleus. In the sex cells the chromatin is uniformly distributed in small granules; in the surrounding cells it is collected in two or three masses. Not all the eggs of this stage show the sex cells equally well. In some eggs in which the blastopore is closing none can be made out with certainty. In some eggs many large cells were seen in the ectoderm but the inner cells of the blastoderm segment more rapidly toward the close of segmentation than the outer cells and these outer ones become distended through intracellular digestion of the surrounding ovarian fluid. But it is still possible that the sex cells arise in the ectoderm. If they are segregated as early as the fifth generation, *i. e.*, before there is any differentiation into ectoderm and entoderm, it seems to be of no great importance whether at the time of the separation of the blastoderm into ectoderm and entoderm the cells lie in its inner or outer portion. There is no change in number or size up to the time the larvæ are 2.5 mm. long. The length over which the majority of cells is distributed is about 0.20 mm.

Eigenmann ('96) continued his observations on the sex cells in *Micrometrus aggregatus.* He finds that the striking feature is the asymmetry of the two sides and the variation in number and position of the cells in different larvæ. He finds the nuclei in the sex cells of embryos 5 mm. in length have become somewhat larger and the nucleoles also so that in the period of apparent inactivity there have been histogenic changes just as truly as the changes in the somatic cells although there is no division. In later stages the tissues containing the sex cells form a median

ridge in the dorsal end of the mesentery. This ridge divides anteriorly into two ridges each of which end in a single little fold of the peritoneum which is differentiated only by being rich in nuclei. In some larvæ at this time the germ cells have not increased in number, in others they may have divided once. In one larva in which a few cells were widely separated from the majority, the ridge entirely disappeared to reappear in the neighborhood of some widely separated anterior cells. From such instances it is evident that the germinal ridge develops only under the influence of the reproductive cells.

Dodds ('10) in *Lophius* was able to recognize germ cells when the blastoderm had not quite half covered the yolk. At this time they are in the primary entoblast. They pass into the mesoblast when this is separated and into that part which becomes the myotome. Later when the mesoblast is separated into myotome and lateral plate the germ cells migrate into the latter and again when this splits they are left in the splanchnopleure. Later they migrate toward the median border of the cœlome and thence upward into the somatic layer and to the position of the permanent germ cells.

The number of germ cells varies but is small—not over fifty-five. From the time they are recognizable until they are in the position of the germ gland there is no increase in number. The apparent increase in early stages is due to changes in the cells which make them more easily recognizable. The cells are characterized by rounded outlines or they may be amœboid. The cytoplasm stains more deeply than that of the somatic cells, the nucleus is smaller, irregular in shape, apparently less turgid and contains two small nucleoles. The nuclei of the somatic cells have two large nucleoles or a single very large nucleole. The decrease in the size of the germ cell nucleoles is due to a loss of part of their content through the nuclear membrane into the cytoplasm. This extrusion of nuclear material does not take place simultaneously from the nucleoles of any one nucleus nor from all germ cells.

There is no segmental arrangement of the germ cells. There is both an active migration of the cells and a passive change due to growth of the surrounding tissue. Dodds is of the opinion

that before any differences between germ cells and somatic cells can be detected there must be an unseen physiological difference which determines the future behavior of the cells.

The eggs of *Amiurus nebulosus* vary in size in the different nests. There is often a difference of 1 mm. in diameter. For this reason it follows that embryos of the same length are not necessarily of the same stage of development. Embryos 3.2 mm. long taken from one nest may be slightly further developed than those 3.7 mm. long taken from another nest.

When stained with iron hematoxylin the yolk granules of the egg are black but if this is washed out with iron alum and then followed by congo red the granules are a deep red. The yolk material in the germ cells is more or less diffused through the cytoplasm and loses the black hematoxylin stain quite readily in the iron alum. But because of the contained yolk the cytoplasm of the germ cells stains a deeper red than the cytoplasm of the surrounding cells. Occasionally there is a small compact mass of yolk in a germ cell which retains the black stain. Yolk granules of the egg stain lightly with Mayer's hæmalum but take up the Orange G very rapidly. Hence the cytoplasm of the germ cells stains a light orange with hæmalum and Orange G. The cytoplasm of the surrounding cells is a bluish gray.

The germ cells in all of the stages studied up to the time of multiplication were found to be about the same size. They are from 14 to 18 microns in diameter and the nuclei 7 to 9 microns in diameter. The germ cells are quite well rounded though sometimes they are more or less amœboid. The cytoplasm is quite clear, especially so if the contained yolk is in one or two compact masses. The nucleus is spherical or frequently lobed. But this lobed condition of the nucleus is not peculiar to the nuclei of germ cells—it is often found in nuclei of other cells. There are often two nucleoles but again this is no peculiarity of germ cell nuclei. In the very early stages of development most of the somatic cells have more than one nucleole and two nucleoles are seen in the nuclei of many somatic cells long after the cœlomic cavity is formed. Not infrequently there are no nucleoles in the germ cell nuclei. The chromatin which is fairly distinct is scattered through the nucleus in the form of fine granules. It

is never aggregated into large masses as is common in the nuclei of somatic cells.

The germ cells with few exceptions have been found in the mesoderm. These exceptions were a few cells in the cœlomic cavity or between the lateral plate of mesoderm and the yolk, also a few doubtful cells in the entoderm. In younger embryos the germ cells are in the lateral plate of the mesoderm and near the region where the blastopore has closed. In older embryos where the tail has grown out beyond the yolk they are in the splanchnopleure surrounding the hind gut. Later they are in the mesentery and in the germ gland anlage. The exact location of the germ cells will be given in greater detail in the description of the different stages studied.

The number of germ cells is constant until after or about the time they are in the germ gland anlage. The average number is about 23 with extremes 12 and 34. Because of the large size of the germ cells and the thickness of the sections it would be possible to count the same cells twice. However, by noting the position and appearance of the cells and the size of the nucleus, it is believed that few errors have been made in counting.

There is probably no one characteristic peculiar to germ cells in *Amiurus nebulosus*. Blood corpuscles are frequently nearly spherical. But in these the cytoplasm stains more lightly with congo red and Orange G., the nucleoles are larger, and the nuclear membrane much more distinct than in the germ cells. Also the ratio of the diameter of the nucleus to the diameter of the cell is greater in blood corpuscles than the same ratio in germ cells. In a number of blood corpuscles this ratio was 2 : 3, in germ cells it is 1 : 2. The cytoplasm of the entoderm cells often contains much yolk and hence these frequently stain like germ cells. But the entoderm cells are usually smaller and columnar or irregular in shape, and the nuclei are more often oval than spherical with two or three large nucleoles. In the earliest stage studied the mesoderm cells were not more than half the size of the germ cells and contained little if any yolk. Even in this stage the nuclei of the mesoderm cells are becoming oval or elongated. In later stages the mesoderm cells are small, very irregular in shape with cytoplasmic processes. The germ cells

are easily recognized in any stage by their shape, size, and staining qualities.

Embryos 3.2–3.7 mm. Long.

In embryos of this length the blastopore is closed and the tail extends about 1 mm. beyond the yolk. The pronephric ducts have been formed. There is no trace of a split in the mesoderm which is to give rise to the cœlomic cavity. The axial mesoderm is a compact mass of cells extending laterally and ventrally and composed of cells which are still quite regular in outline although the nuclei are more or less oval. There is scarcely a trace of yolk in these mesodermal cells. The entoderm cells frequently contain large yolk masses or have yolk material diffused throughout the cytoplasm.

The germ cells are found in the lateral plate of mesoderm in the region just anterior to where the tail leaves the yolk. The germ cells may be anywhere in the lateral plate but the majority of them were found some distance away from the mid-line (Fig. 1). Not infrequently they are far out in the lateral plate as shown in Fig. 2. A few germ cells were found between the yolk and the mesoderm. It may be possible that they came from the yolk but at least none were seen in the yolk nor coming out of it. The germ cells are so very different in appearance from the other somatic cells that there is no danger in overlooking them in well-stained sections. The length of the region in which they are found varies from 0.025 mm. to 0.054 mm. The embryos are not symmetrical with regard to the number of germ cells found on each side. The total number of an embryo is from 12 to 28. The following table shows the number found on each side, the total number, and the distance throughout which the germ cells were distributed in several of the embryos sectioned.

	Right.	Left.	Total.	Length of Region, Mm.
1.....	15	8	23	0.250
2.....	8	14	22	0.260
3.....	10	9	19	0.360
4.....	14	13	28	0.250
5.....	4	9	13	0.340
6.....	12	13	26	0.640

In number 4, one cell was in the entoderm, and in number 6, a germ cell was between the yolk and the mesoderm.

Embryos 5 mm. Long.

The cœlomic cavity is completely formed. With the growth in length a greater differentiation in tissues has been going on. The germ cells are found in the region of the hind gut, the majority of them are posterior to where the tail leaves the yolk. The most anterior cells are 0.080 to 0.220 mm. anterior to this region. The most posterior cells were 0.180 to 0.290 mm. anterior to the anus. With few exceptions the germ cells were found in the splanchnopleure (Fig. 3). In one embryo a nest of four cells was found in the somatopleure (Fig. 4). In the same embryo a germ cell was found lying in the center of the cavity between the somatopleure and the ectoderm. This was such an unusual position that it seems reasonable to think that it might have been torn away from the somatopleure in sectioning; however, the section has not the appearance of having been torn in any way. The size and appearance of the germ cells of embryos 5 mm. long are the same as in the preceding stage, but they are much more conspicuous due to the greater differentiation of the surrounding mesoderm. The cells are in the dorsal half of the splanchnopleure and often give the appearance of crowding toward the mesentery next to the alimentary tract (Fig. 5). The germ cells are distributed throughout a distance averaging 0.420 mm. in length. The following table shows in three embryos the unsymmetrical arrangement, the number of cells, the length of region throughout which the germ cells are distributed, the distance anterior to the anus of the most posterior germ cell and the distance anterior to the region where the tail leaves the yolk

	R.	L.	Total.	Length of Germ Cell Region, mm.	Dis. Ant. to Anus, mm.	Dis. Ant. to Tail, mm.
1.	6	26	32	0.420	0.100	0.230
2.	6	17	25	0.450	0.180	0.100
3.	9	7	22	0.400	0.290	0.220

of the most anterior germ cell. In embryo number 2, 2 germ cells were in the mesentery; 6 cells were in the mesentery in number 3.

EMBRYOS 7 MM. LONG.

Three embryos of this length were sectioned. In one of these there were 26 germ cells most of which were in the mesentery or at least very near it (Fig. 6). In the other two embryos the germ cells had probably divided once although there was little difference in the size of the cells from those in earlier stages. The cells were 14–16 microns in diameter and the nuclei 7–8 microns. There were 57 cells in one embryo and 63 in the other. In both embryos most of the germ cells were crowded in the mesentery in nests of 3 to 8 (Fig. 7). A few single cells were nearer the germ gland anlage or still in the splanchnopleure. Except in the case of one cell dividing, the appearance of the nuclei, cytoplasm, etc., seemed the same as in cells in earlier stages. The germ cells are in the same region as in the 5 mm. stage. The following table gives the number of cells, the length throughout which the cells were distributed, the distance anterior to the anus of the most posterior cell, and the distance anterior to the tail region of the most anterior cell.

	Total No.	Length of Region, Mm.	Dis. from Anus, Mm.	Dis. from Tail, Mm.
1.	26	0.680	0.120	0.220
2.	63	0.400	0.210	0.240
3.	57	0.430	0.120	0.320

EMBRYOS 9 MM. LONG.

In embryos of this length the anlagen of the germ glands appear as a fold in the peritoneum on either side of the base of the mesentery. (Figs. 8 and 9). This fold extends from 0.030 to 0.280 mm. anterior to the most anterior germ cell. In one embryo the fold was poorly developed for a distance of 0.080 mm. where there were no germ cells but this is not the most common condition. After the fold once appears, it is continuous throughout the region in which the germ cells are found. In one embryo there were no germ cells for a distance of 0.200 mm. but in this entire distance the fold of the peritoneum was very prominent. The fold seems to extend as far posterior from the germ cell region. It was found to extend 0.080 to 0.230 mm. posterior from the most posterior cell. In all of the embryos of this stage which

were sectioned the germ cells had found their way into the peritoneal fold. The number remains the same—28 to 30. The size and appearance of the cells agree exactly with the same in the earliest stages. The germ cells are distributed throughout a distance of 0.980 to 1.020 mm. The most posterior cells are 0.420 to 0.520 mm. anterior to the anus. It will be seen that although the embryos have increased much in size, the length throughout which the germ cells are distributed in proportion to the length of the embryo is about the same.

Embryos 14 mm. Long.

In all of the embryos of this stage the germ cells had divided at least twice. There had probably been two divisions in one embryo where the number was 144. Here the germ cells measure 8–12 microns in diameter and the nuclei are 4–6 microns. The chromatin is no longer in such fine granules as in the early stages but is often aggregated into small masses. The germ cells are in the center in the peritoneal fold with strands of peritoneal cells between them (Fig. 10). There is nothing which would suggest a differentiation of peritoneal cells into germ cells. Some of the germ cells are smaller than others but it is believed that these are cells which have divided more times than the larger ones and not that they are transition cells (see Fig. 10). No germ cells were found as large as even the smallest of those in younger stages. The germ cells and peritoneal cells stain as distinctly different as described before and no cells intermediate in size or staining qualities were found. In this embryo of 144 germ cells the germ gland or fold extends only 0.010 mm. anterior to the first germ cell but 0.500 mm. posterior from the most posterior germ cell. The germ cells were distributed throughout a distance of 1.280 mm. and the germinal fold throughout 1.790 mm.

In another embryo of the same length the germ cells had evidently divided several times—the cells here were only 6 or 7 microns in diameter and the nuclei about 34 in diameter. There are twenty or more germ cells in every section of each of the glands. The cells were not counted in the entire embryo. They are distributed throughout a distance of 1.220 mm. The most

posterior germ cell is 0.040 mm. posterior to the anus and the germ gland extends 0.160 mm. posterior to the anus. The germinal fold extends only 0.050 mm. anterior to the first germ cell.

Conclusions.

Germ cells in *Amiurus nebulosus* are distinct from all other cells at least from the time the embryo is 3.2 mm. long. They are then in the lateral plate of the mesoderm. The number of germ cells varies in the different embryos. Until the time when these cells are in or near the germ gland anlage, the average number is 23. With respect to the number of germ cells found on each side the embryo is not symmetrical. When the cœlome is formed they are largely in the splanchnopleure in which they migrate toward the mesentery and in this to the germ gland anlage. There is in most cases no multiplication in number of cells up to the time the germinal fold is formed. The cells migrate into this fold and then divide. There was no transition of peritoneal cells into germ cells observed in any of the stages studied. The epithelial covering and the stroma of the germ gland is derived from peritoneal cells. The germinal fold develops in regions where there are no germ cells.

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EXPLANATIONS OF THE FIGURES.

All figures were outlined with the aid of a camera lucida.

PLATE I.

FIGS. 1 AND 2. Germ cells in lateral part of mesoderm. × 390.

FIG. 3. Formation of cœlome. Germ cells in splanchnic layer. × 390.

FIG. 4. A nest of germ cells in somatopleure. × 390.

FIG. 5. Germ cells migrating toward mesentery. × 390.

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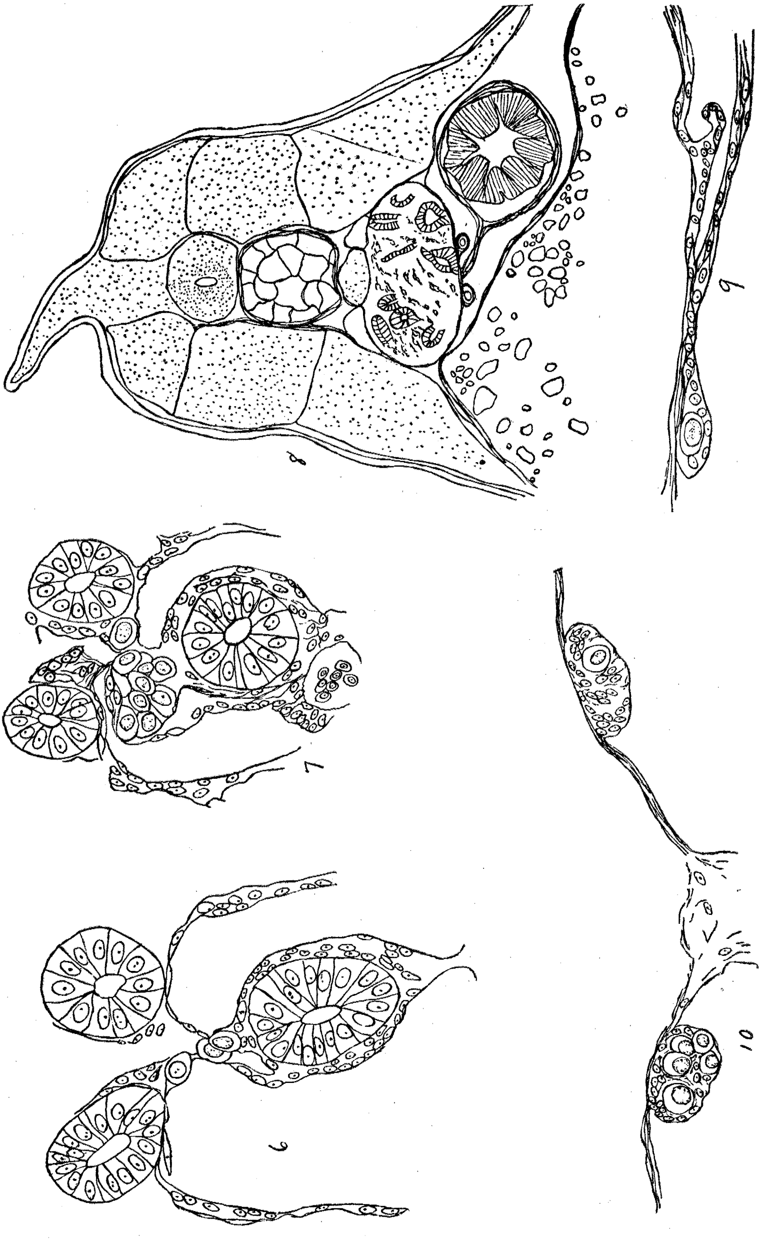
PLATE II.

FIGS. 6 AND 7. Germ cells migrating dorsally in mesentery. $\times$ 390.

FIG. 8. Diagrammatic representation of cross section of embryo to show the relative position of the germ glands. $\times$ 72.

FIG. 9. The origin of the germ gland from a fold in the peritoneum. $\times$ 390.

FIG. 10. Germ cells within the germ glands. $\times$ 390.



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